

Immunity, Volume 48

Supplemental Information

Transformation of Accessible

Chromatin and 3D Nucleome Underlies

Lineage Commitment of Early T Cells

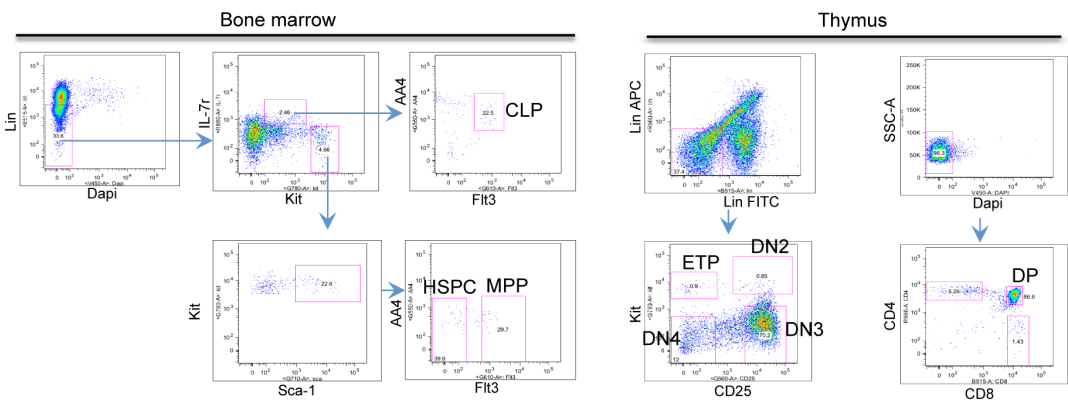
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Supplementary Information

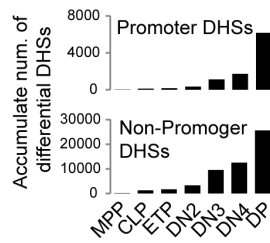
Supplementary FIGURES

Figure S1

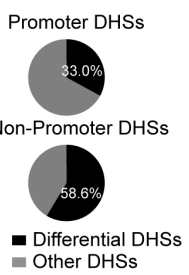
A



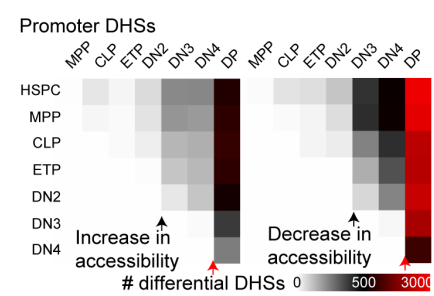
B



C



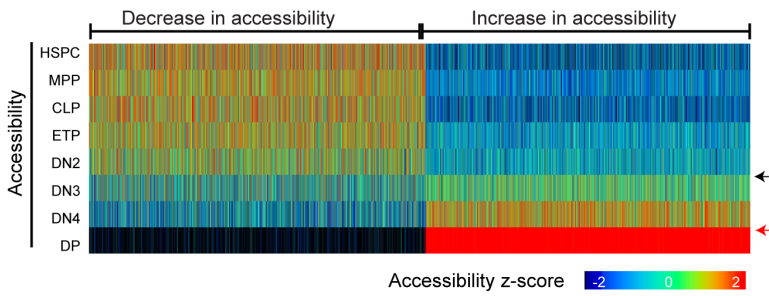
D



E

Diff. accessibility from DN4 to DP	Diff. accessibility from HSPC to DP	# DHSs	GO BP enrichment analysis by GREAT (FDR < 0.01; FC > 2; top 5, if any)	Motif enrichment analysis by HOMER (p < 1e-10; top 5, if any)
NO	Concordant increase	4070	lymphocyte differentiation; cell activation; lymphocyte activation; epithelial cell morphogenesis; antigen receptor-mediated signaling pathway	CAGCACCTGC (E2A); ACCACATCAAAG (TCF7); TTGTCTTTGA (TCF7); GTGGTTTG (RUNX1); CAGCTGCACA (GM397)
NO	Concordant decrease	6528	angiogenesis; cellular response to vascular endothelial growth factor stimulus; negative regulation of MAP kinase activity; vascular endothelial growth factor receptor signaling pathway; mesoderm morphogenesis	AAAAGAGGAAGT (PU.1)
YES	Concordant increase	6493	lymphocyte differentiation; antigen receptor-mediated signaling pathway; regulation of interleukin-2 production	TGACCTACTTTC (RORYt); ACAGGAAGTGGT (ETS); ACATCAAAGA (TCF7); ACATCAAAGA (DMRT3); AGTACTTCTAAA (NKX2-9)
YES	Concordant decrease	6636	mesoderm development; cell fate commitment; mesoderm morphogenesis; platelet-derived growth factor receptor signaling pathway; artery morphogenesis	NO HIT

F



G

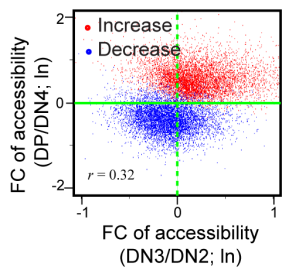


Figure S1: Change of chromatin accessibility at the DN4-to-DP transition follows that at the DN2-to-DN3 transition – **related to Figure 1**

- (A) FACS profiles for isolating HSPC, MPP and CLP cells from bone marrow and for isolating ETP, DN2, DN3, DN4, and DP cells from thymus.
- (B) Accumulative number of differential DHSs in promoter regions (upper panel) and non-promoter regions (lower panel) from HSPC to flowing stages from MPP to DP cells.
- (C) Pie graph showing the distribution of differential DHSs in promoter regions (upper panel) and non-promoter regions (lower panel).
- (D) Heat map showing the numbers of differential DHSs in promoter regions, increasing (left panel) or decreasing (right panel) in chromatin accessibility, for any two stages from HSPC to DP. Black and red arrow heads: DN2-to-DN3 transition and the DN4-to-DP transition, respectively.
- (E) GREAT Gene Ontology enrichment analysis and HOMER motif enrichment analysis for DHSs with concordant change in accessibility from HSPC to DP, sorted first based on the change in accessibility at the DN4-to-DP transition and then by the direction of concordant change in accessibility across all stages from HSPC to DP cells.
- (F) Heat map visualization of scDNase-Seq read density for non-promoter DHSs that show differential accessibility between DN4 and DP cells, sorted based on concordant increase or decrease in chromatin accessibility from HSPC to DP cells. Black and red arrowheads: the DN2-to-DN3 transition and the DN4-to-DP transition, respectively.
- (G) Scatter plot for the change in the z-score of scDNase-Seq read density at DN2-to-DN3 transition and at the DN4-to-DP transition for the same group of DHSs from panel F. r : Pearson's correlation coefficient.

Figure S2

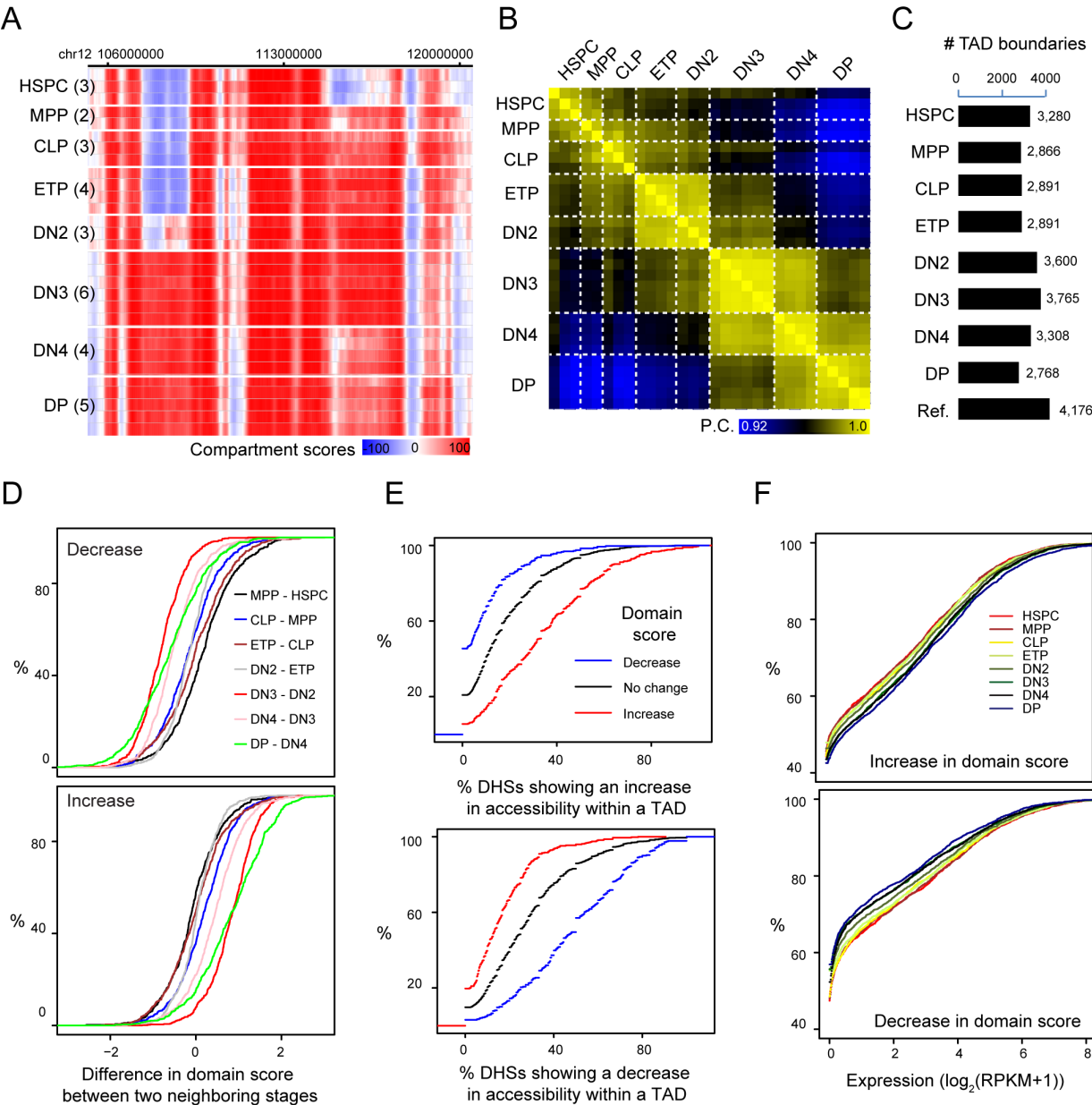


Figure S2: Coordinated change of intra-TAD connectivity and chromatin accessibility at enclosing DHSs – **related to Figure 2**

- (A) Heat map showing the distribution of compartment score across a 14M bps genomic region in chromosome 12 for all replicates of Hi-C libraries prepared for each developmental stage from HSPC to DP. Number in parentheses: number of replicates for each stage.
- (B) Heat map showing the Pearson's correlation coefficients of compartment scores across all 20K bps genomic bins for a pair-wise comparison of all Hi-C libraries.
- (C) Numbers of TAD boundaries identified for each developmental stage and number of TAD boundaries pooled from all stages, termed as the reference boundaries.
- (D) Empirical cumulative distributions of the difference in z-score of domain scores between every two consecutive stages for TAD showing a decrease (upper panel) or increase (lower panel) in domain scores. Y-axis records the % of TAD with a difference in domain score lower than the one when specified by the X-axis.
- (E) Empirical cumulative distribution of the % of DHSs that show increase (top panel) or decrease (bottom panel) in accessibility within TADs for TADs sorted based on the change of domain score from HSPC to DP.
- (F) Empirical cumulative distribution of expression values for genes extracted from TADs with an increase (upper panel) or decrease (lower panel) in domain scores.

Figure S3

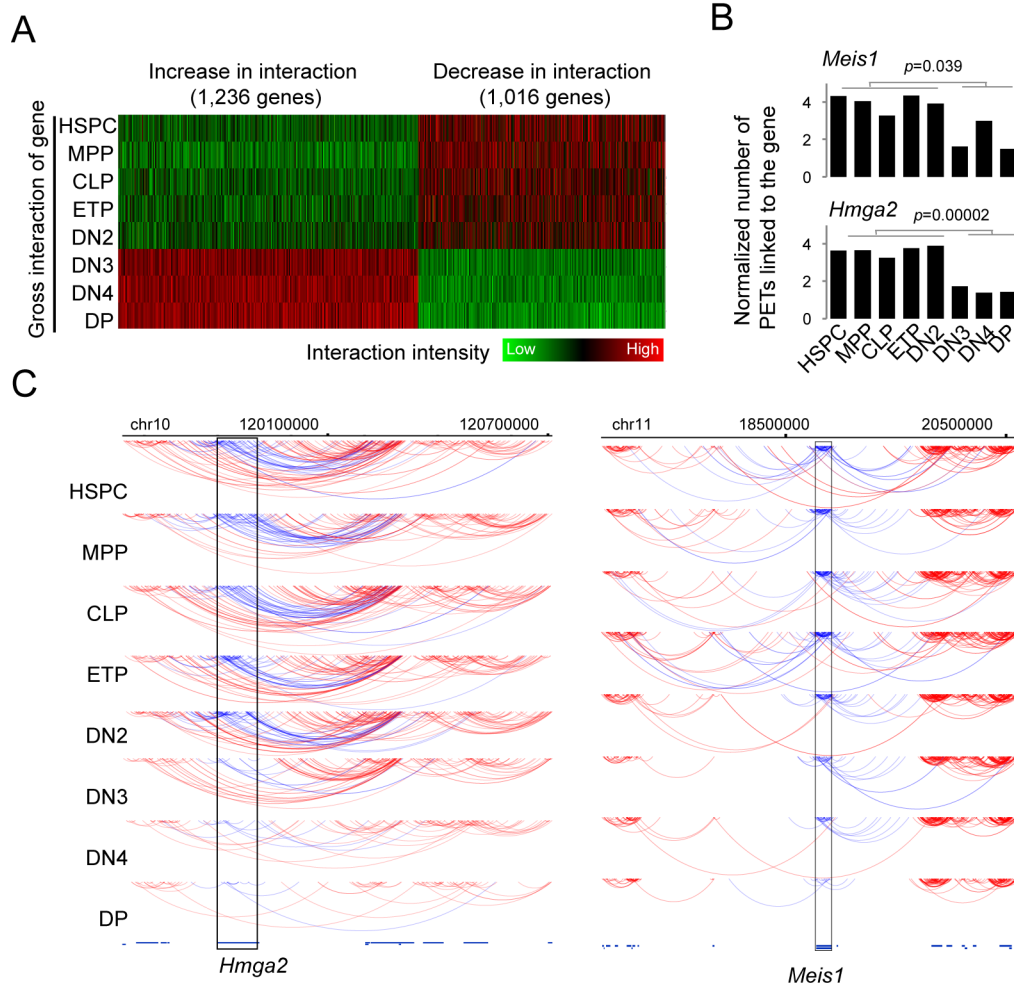


Figure S3: Genes with a substantial decrease in interaction are gradually silenced – related to Figure 3

- (A)** Heat map of interaction density from HSPC to the DP stage for genes that showed significant increase or decrease in the number of gross interaction before and after T cell commitment.
- (B)** Bar graphs showing examples of genes with a substantial decrease (*Hmga2* and *Meis1*) in the number of gross interacting PETs linked to the genes before and after T cell commitment. *P*-value by *t*-test.
- (C)** Arc plot from WashU epigenome browser showing the gross interacting PETs of *Hmga2* (left panel) and *Meis1* (right panel) from HSPC to DP cells. Blue lines: PETs with at least one end linking to *Hmga2* (or *Meis1*) locus (indicated by rectangles). Pooled from 2-5 independent experiments.

Figure S4

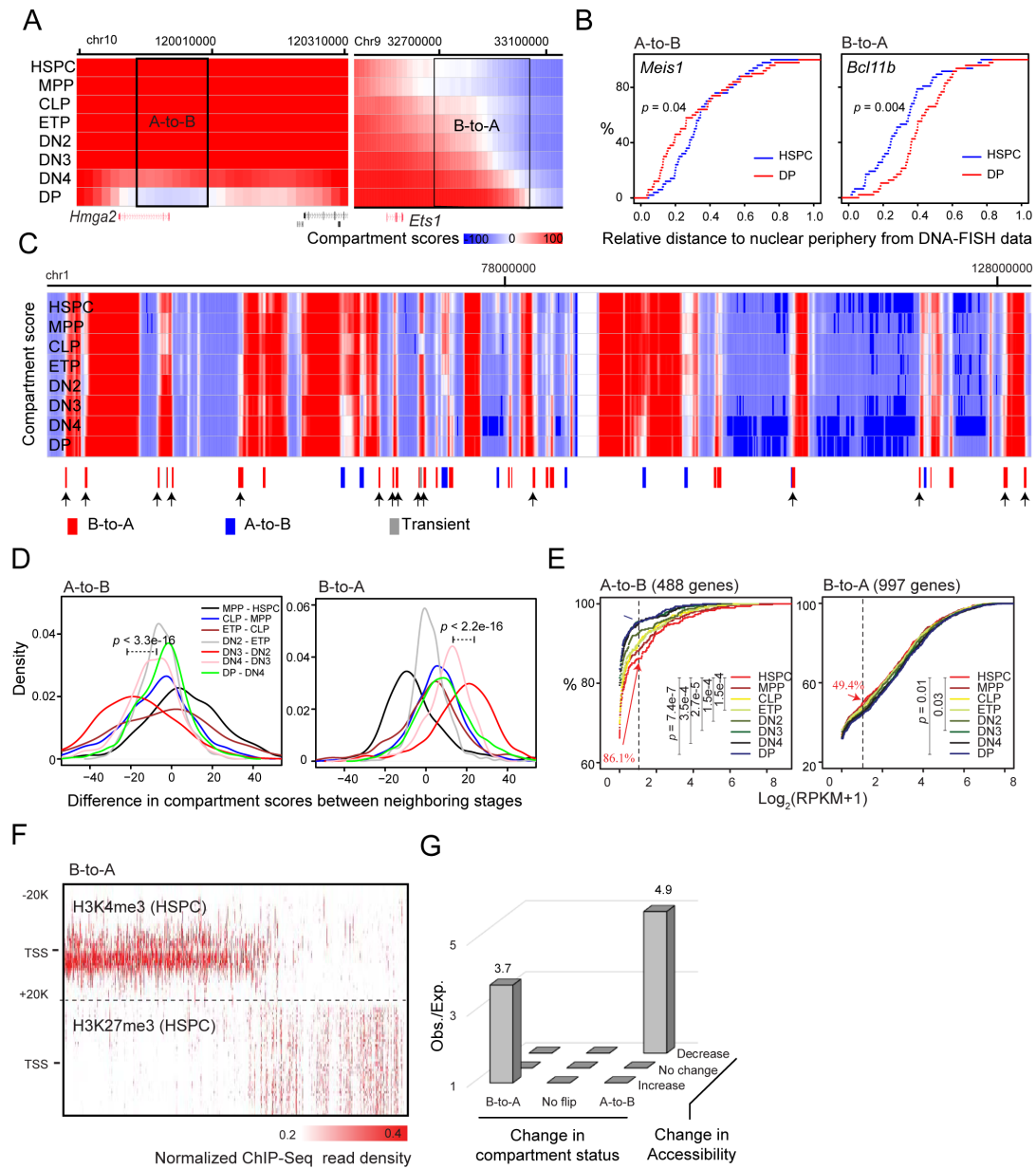


Figure S4: Correlation of compartment reorganization and change in expression and chromatin states – **related to Figure 4**

- (A) Heat maps showing the distributions of compartment score across genomic regions encompassing *Hmga2* (left panel) and *Ets1* (right panel), for each developmental stage from HSPC to DP. Rectangles: genomic regions with a compartment flip. Pooled from 2-5 independent experiments (also applied to C).
- (B) Empirical cumulative distribution of the relative distance to nuclear periphery for *Meis1* locus (left panel) and *Bcl11b* locus (right panel) in HSPC cells (blue line; 28 cells) and in DP cells (red line; 30 cells), with a line shifting to the left side indicating a closer distance to the periphery. *P*-value by Kolmogorov-Smirnov test.
- (C) Heat maps showing the distributions of compartment score across a 100M genomic region in chromosome 1, with flipped compartment regions indicated. Arrow heads: regions with B-to-A flip occurring at compartment boundaries.
- (D) Distribution of the difference in compartment scores between every two neighboring stages for genomic regions showing concordant A-to-B compartment flips (left panel) and concordant B-to-A compartment flips (right panel). *P*-value by Kolmogorov-Smirnov test.
- (E) Empirical cumulative distribution of expression values for genes extracted from A-to-B flipped compartments (left panel) or from B-to-A flipped compartments (right panel) for each developmental stage. Y-axis: the percentage of genes with expression values lower than the one when specified by the X-axis. A line shifting to the left side corresponds to a generally lower expression within the population. Dotted vertical line: an RPKM value of 1, a cutoff to separate expressed and non-expressed genes. *P*-value by Kolmogorov-Smirnov test.
- (F) Distribution of active histone modification H3K4me3 and repressive histone modification H3K27me3 in HSPC cells surrounding TSS (± 20 K bps) of genes extracted from B-to-A flipped compartments.
- (G) 3D bar graphs for the ratios of observed (Obs.) to expected (Exp.) number for DHSs grouped based on their change in chromatin accessibility at the DN2-to-DN3 transition and their change in compartment status during early T cell development.

Figure S5

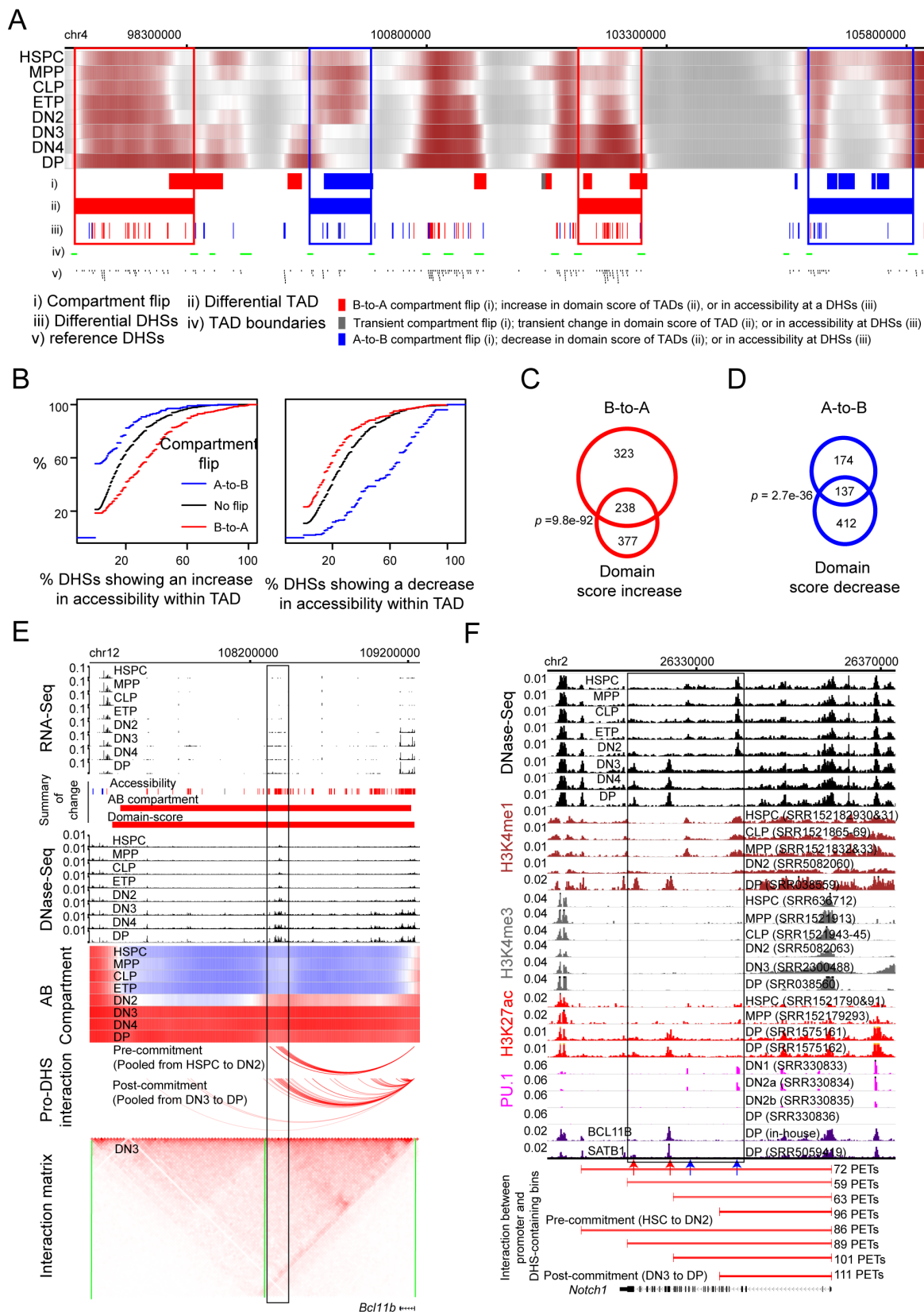


Figure S5: Coordinated change of A/B compartments, TAD connectivity and DHS accessibility – related to Figure 5

- (A) An integrative view of compartment flips (panel i), change in intra-TAD connectivity (panel ii), and change in chromatin accessibility at DHSs (panel iii) for a ~9.5M genomic region in chromosome 4. Top panel: heat maps for the distribution of compartment scores for each stage, pooled from 2-5 independent experiments; panel iv) positions of the reference TAD boundaries; v) positions of the reference DHSs. Red rectangles: TADs that overlap with B-to-A flipped compartments, show an increase in domain score, and harbor DHSs showing a general increase in accessibility. Blue rectangles: TADs that overlap with A-to-B flipped compartments, show a decrease in domain score, and harbor DHSs showing an overall decrease in accessibility.
- (B) Empirical cumulative distribution of the % of DHSs that show increase (left panel) or decrease (right panel) in accessibility within TADs. Three groups of TADs are compared: those overlapping with B-to-A flipped compartments, those overlapping with A-to-B compartments and those showing no compartment flip.
- (C) Venn diagram for the numbers of TADs that show both an overlap with B-to-A flipped compartment and an increase in domain scores (the shared part), or show only an overlap with B-to-A flipped compartment (top part) or show only an increase in domain score (bottom part). *P*-value by binomial test.
- (D) Venn diagram for the numbers of TADs that show both an overlap with A-to-B flipped compartment and a decrease in domain scores (the shared part), or show only an overlap with A-to-B flipped compartment (top part) or show only a decrease in domain score (bottom part). *P*-value by binomial test.
- (E) Online WashU genome browser session showing tracks for RNA-Seq reads (n=2), DNase-Seq reads (n=2), and A/B compartment score (pooled from n = 2-5) for a genomic region surrounding a super-enhancer of *Bcl11b* (rectangle) from HSPCs to DP. Summary of change: tracks that summarizes change in accessibility at DHSs, A/B compartmental status, and domain score within TADs with color encoded the same as that of panel i-iii in A. Green lines: TAD boundaries; arc plots: Fit-HiC predicted interaction between *Bcl11b* promoter and DHS-containing genomic bins (q-value < 0.01). The predictions were made at a resolution of 10K bp for stages pooled from HSC to DN2 (pre-commitment) and for stages pooled from DN3 to DP (post-commitment) to increase statistic power (also applied to panel F). Interaction matrix: shown only for DN2 to save space. n: number of independent experiments;
- (F) Online WashU genome browser session showing tracks for DNase-Seq data (number of independent experiments = 2), representative ChIP-Seq data of H3K4me1, H3K4me2, H3K4me3, H3K27ac, PU.1, BCL11B and SATB1 for stages where data is available, and Fit-HiC predicted interaction (q-value < 0.01) between DHS-containing bins and *Notch1* promoter at a resolution of 10Kbp for stages pre-commitment (pooled from HSC to DN2) and post-commitment (pooled from DN3 to DP). Red arrow heads: DHSs increasing in

accessibility; Blue arrow heads: DHSs decreasing in accessibility; Black rectangle: region of enhancer switch; parenthesis: accession number for public data.

Figure S6

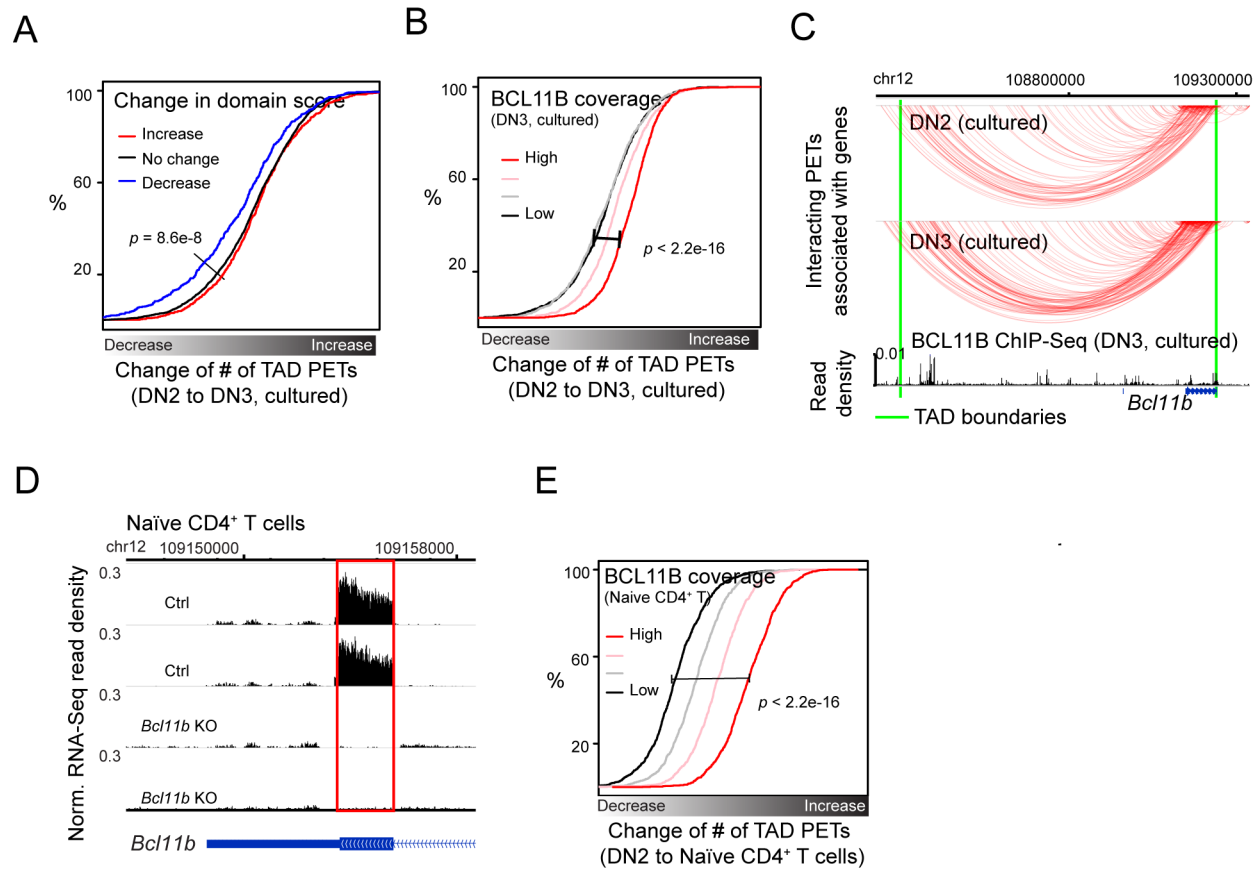


Figure S6: Increase of intra-TAD connectivity is associated with BCL11B binding – related to Figures 6&7

- (A) Empirical cumulative distribution of the fold change of the number of all TAD PETs from DN2 to DN3 cells, both bone marrow-derived cultured for TADs sorted based on their change in intra-TAD connectivity (or domain score) during early T cells development from HSCs to DPs (primary cells). *P*-value by Kolmogorov-Smirnov test (also applied to panels B and E).
- (B) Empirical cumulative distribution of the fold change of the number of all TAD PETs from DN2 to DN3 cells, both bone marrow-derived cultured for TADs sorted into four equal size groups based on their BCL11B coverages in the cultured DN3 cells.
- (C) WashU genome browser showing the distribution of TAD PETs in BM-derived DN2 and DN3 cells (one independent experiment) and the distribution of BCL11B ChIP-Seq reads in BM-derived DN3 for a TAD enclosing *Bcl11b*. Green lines: TAD boundaries.
- (D) WashU genome browser image showing the RNA-Seq read distribution surrounding the last exon of *Bcl11b*, targeted for deletion (highlighted red in rectangle), in control and *Bcl11b* deleted Naïve CD4⁺ T cells.
- (E) Empirical cumulative distribution of the fold change of the number of TAD PETs from DN2 to Naïve CD4⁺ T cells for TADs sorted into four equal size groups based on their BCL11B coverages in DPs.